

Cationic Phospholipid Derivatives

Lipoplexes, lipid mixtures
and bilayer fusion

Robert MacDonald (Northwestern University)

Cationic phospholipid derivatives form complexes with DNA that efficiently deliver DNA to cells.

Two recently-recognized phenomena will be discussed:

- The relationship between lipid phases formed between cationic lipoids and anionic lipids and the efficiency of the cationic derivative as a transfection agent.
- The “mixed cationic lipid effect”: A mixture of a two cationic lipoids with different tails transfect DNA better than either separately.

Acknowledgements

Li Wang

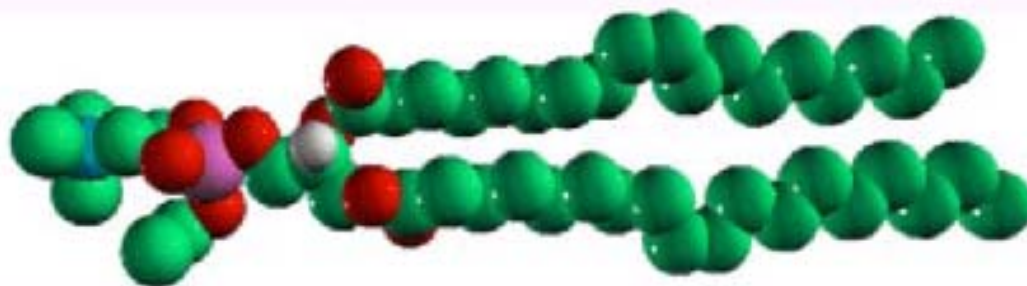
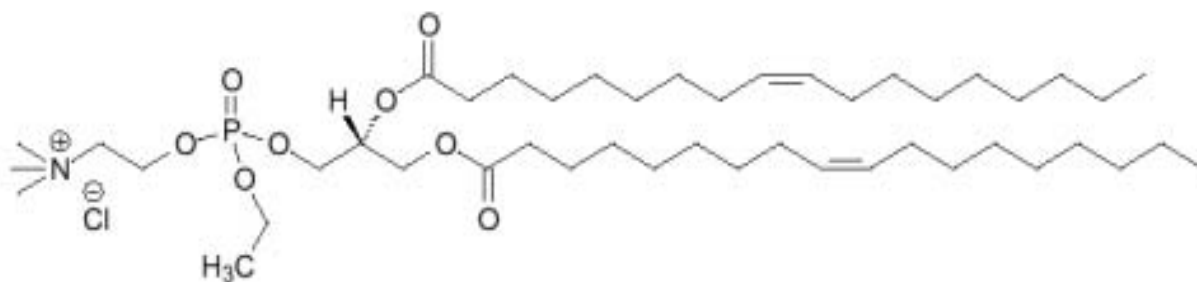
Rumiana Koynova

Yury Tarahovsky

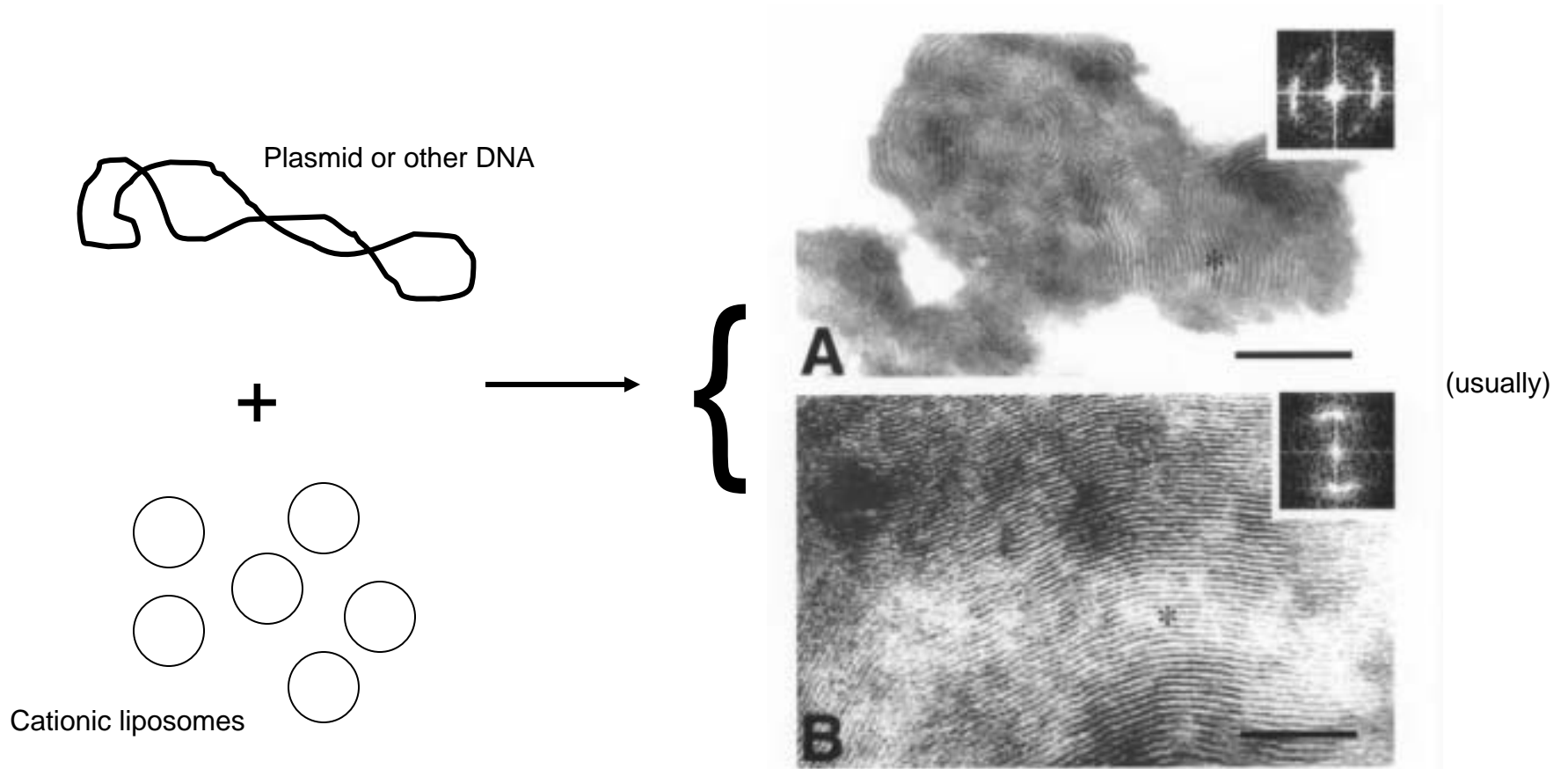
Yaeko Hiyama

CATIONIC PHOSPHOLIPOIDS ARE PHOSPHATIDYLCHOLINE DERIVATIVES

ETHYL PC⁺



LIPOPLEX FORMATION



Lipoplex entry into cells is
nothing complicated

They are gobbled up whole*

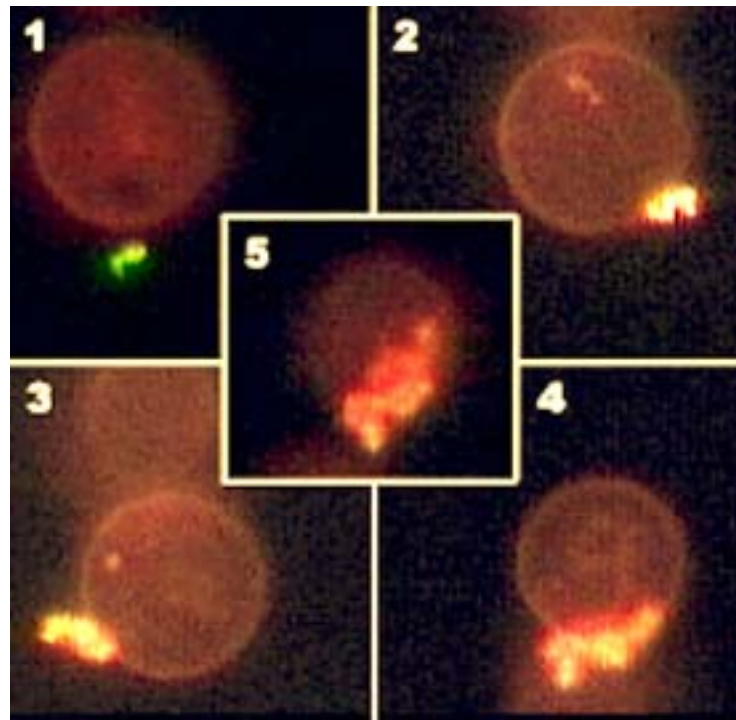
* i.e., “endocytosed”

So, the lipoplex gets into the cell easily, but how does the DNA get out of the lipoplex?

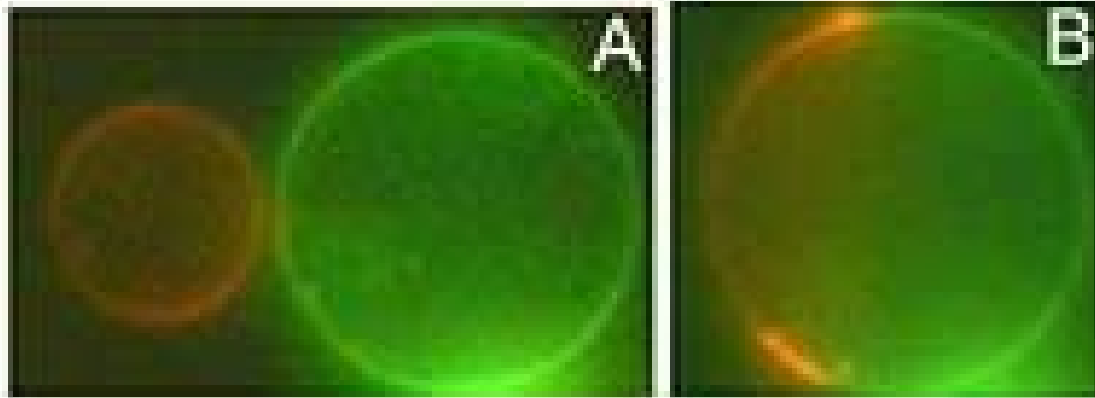
The ΔG of dissociation of DNA from cationic lipids is about 1 kT per nucleotide, so for a several kb plasmid, the extent of dissociation is negligible.

The escape of DNA from the lipoplex must involve neutralization of cationic lipid by cellular anionic lipid, probably by fusion of cell membranes with lipoplexes

When lipoplexes fuse with anionic vesicles, the DNA expands



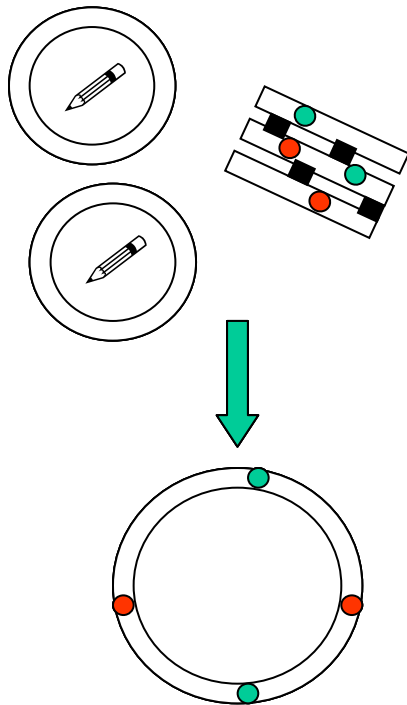
Cationic and anionic lipid bilayers fuse when giant vesicles come into contact



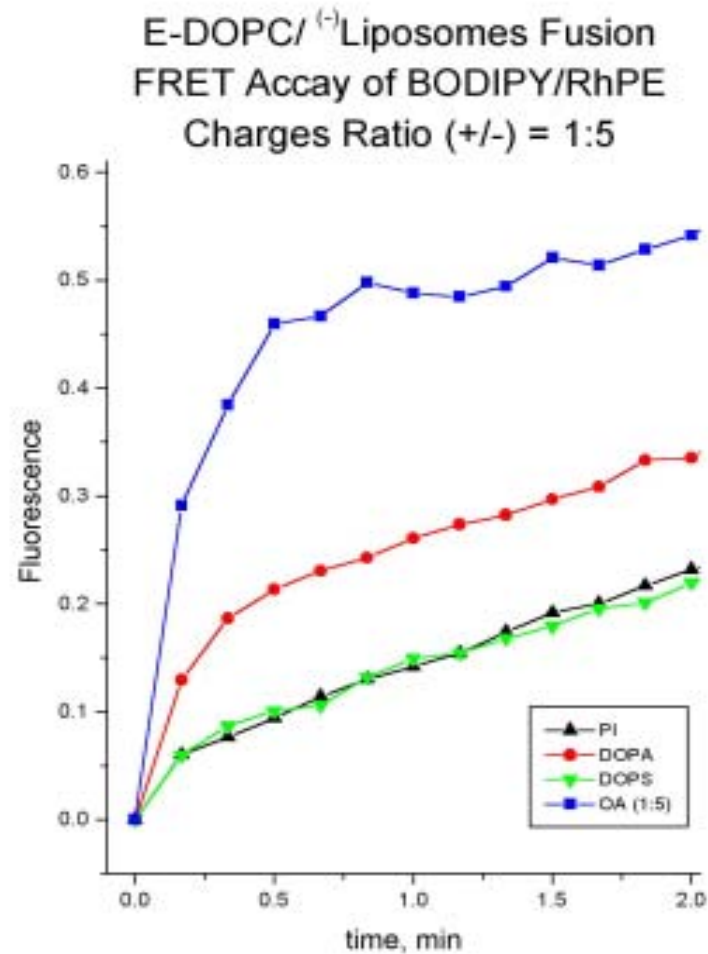
Quantifying effects of anionic lipids on lipoplexes

- Dissociation of DNA from lipoplex lipid was measured with an energy transfer assay (lipid label to DNA label)
- Mixing of the anionic lipid with the cationic lipid of the lipoplex was also measured by an energy transfer assay (lipid label to lipid label)

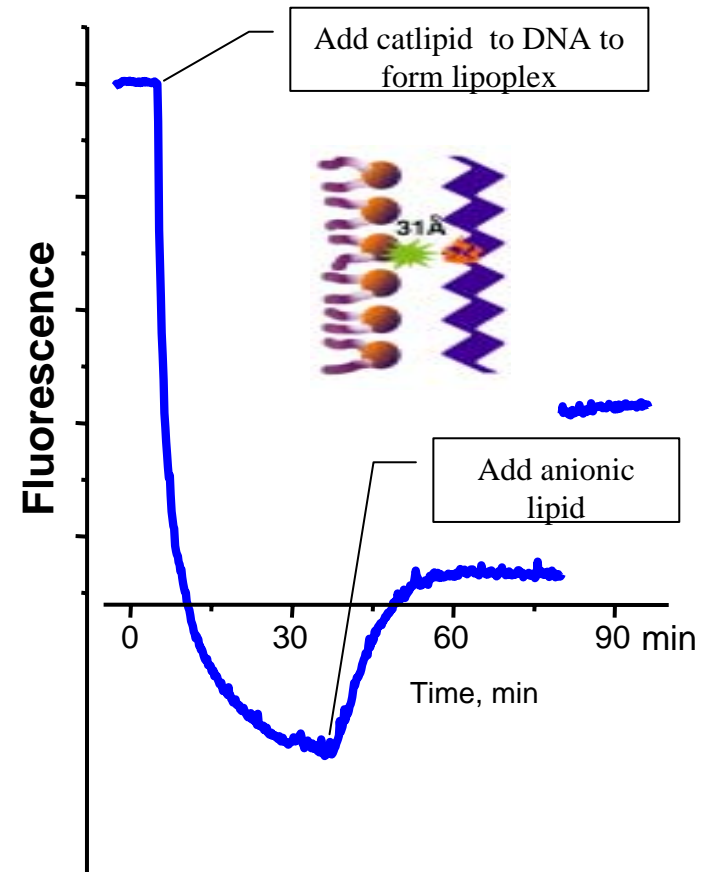
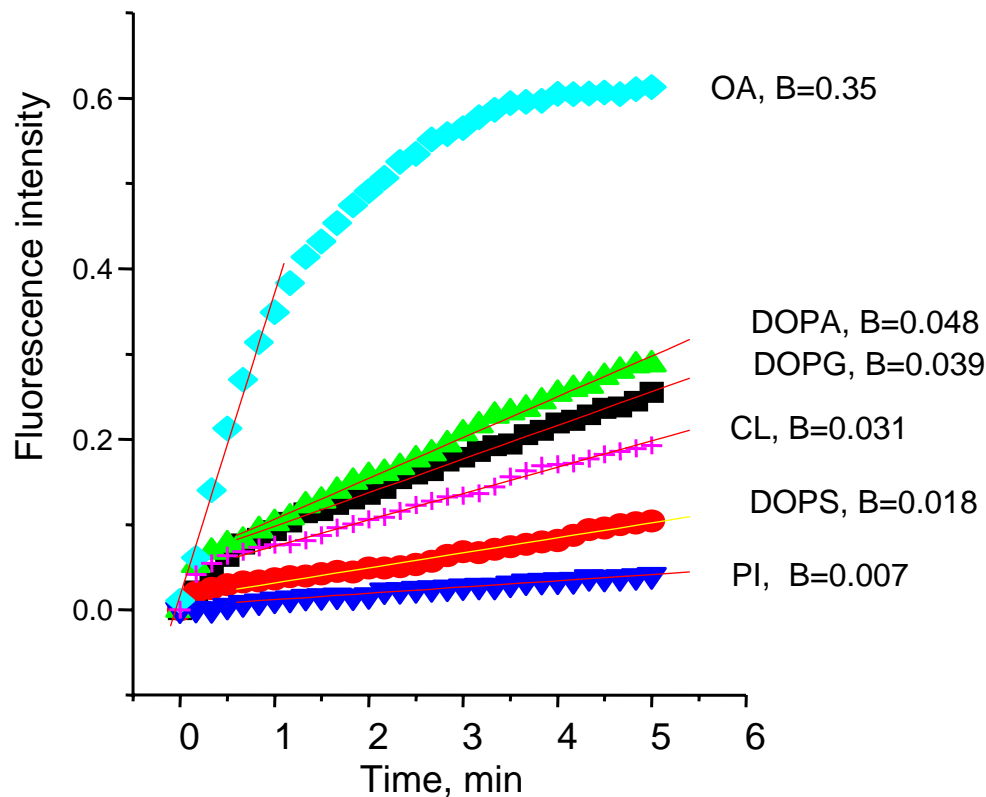
Different anionic liposomes fuse with lipoplex lipid at different rates.



FRET-based assay



When lipoplexes are treated with anionic lipids, the initial rate of separation of cationic lipid and DNA differs, depending on the anionic lipid



What else is involved in DNA release?

The faster the fusion, the faster the neutralization of the cationic charge by anionic lipid and the faster the unbinding of DNA, but, does that necessarily imply complete DNA release from the lipoplex?

No.

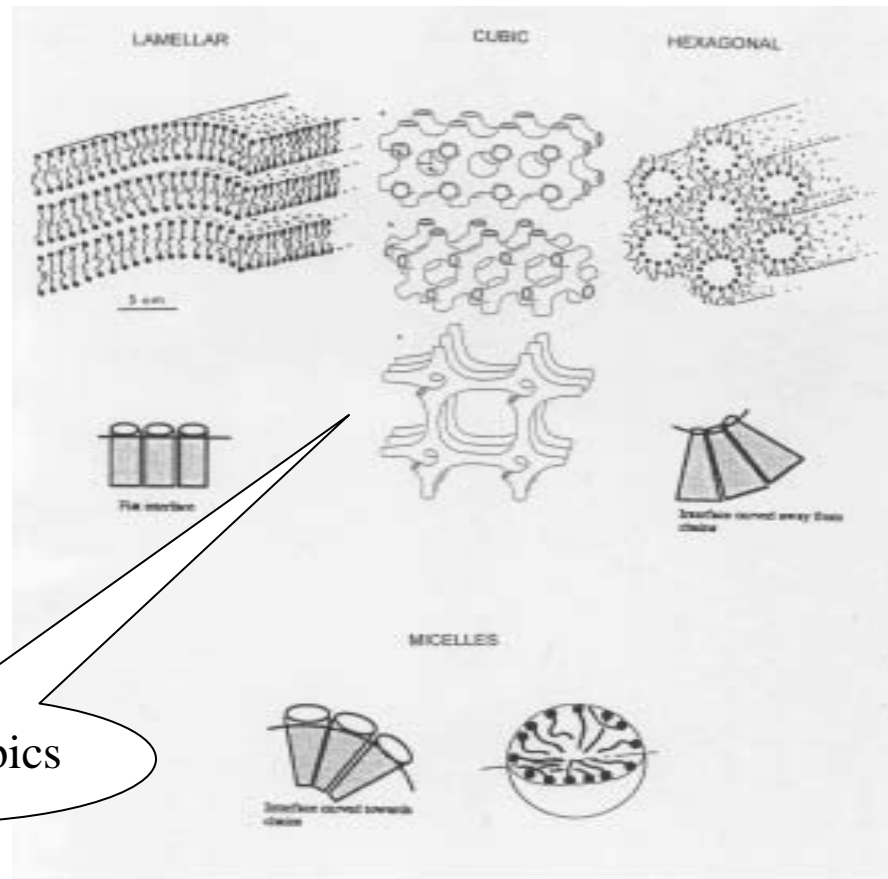
Release will depend on the phase of the lipids, e.g., if they reform into liposomes, the DNA could be encapsulated and would not be free even though it may detach from the bilayer.

.....so let's look at some lipid phases

Some lipid phases

Lipid phase stability depends primarily on molecular shape.

Equal size heads and tails are needed for lamellar arrays. As heads get smaller, cubic and then inverted hexagonal phases become preferred.



Bilayer cubics

Lipid phases as f(intrinsic curvature)

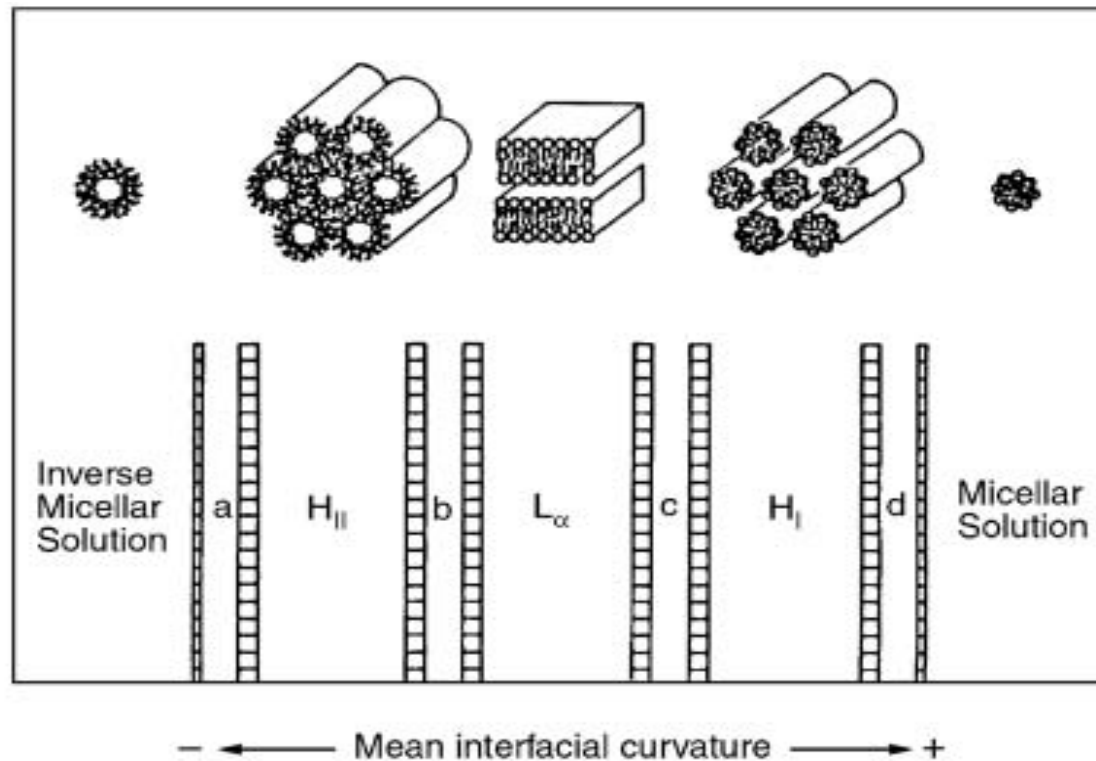
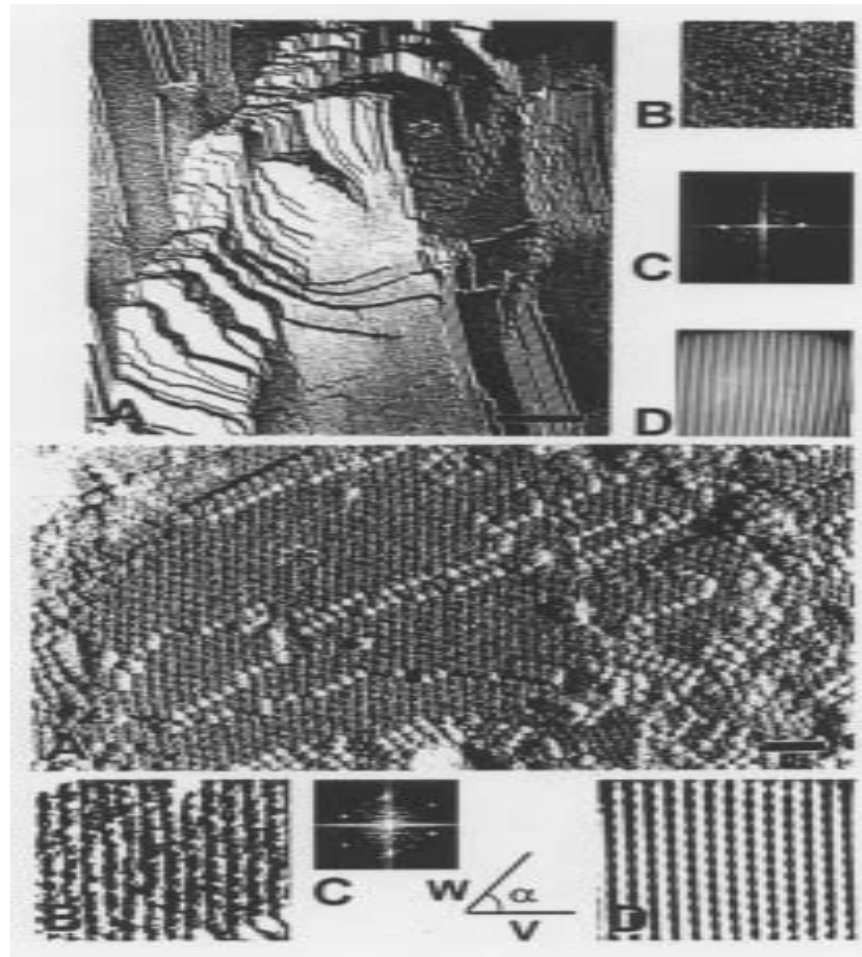


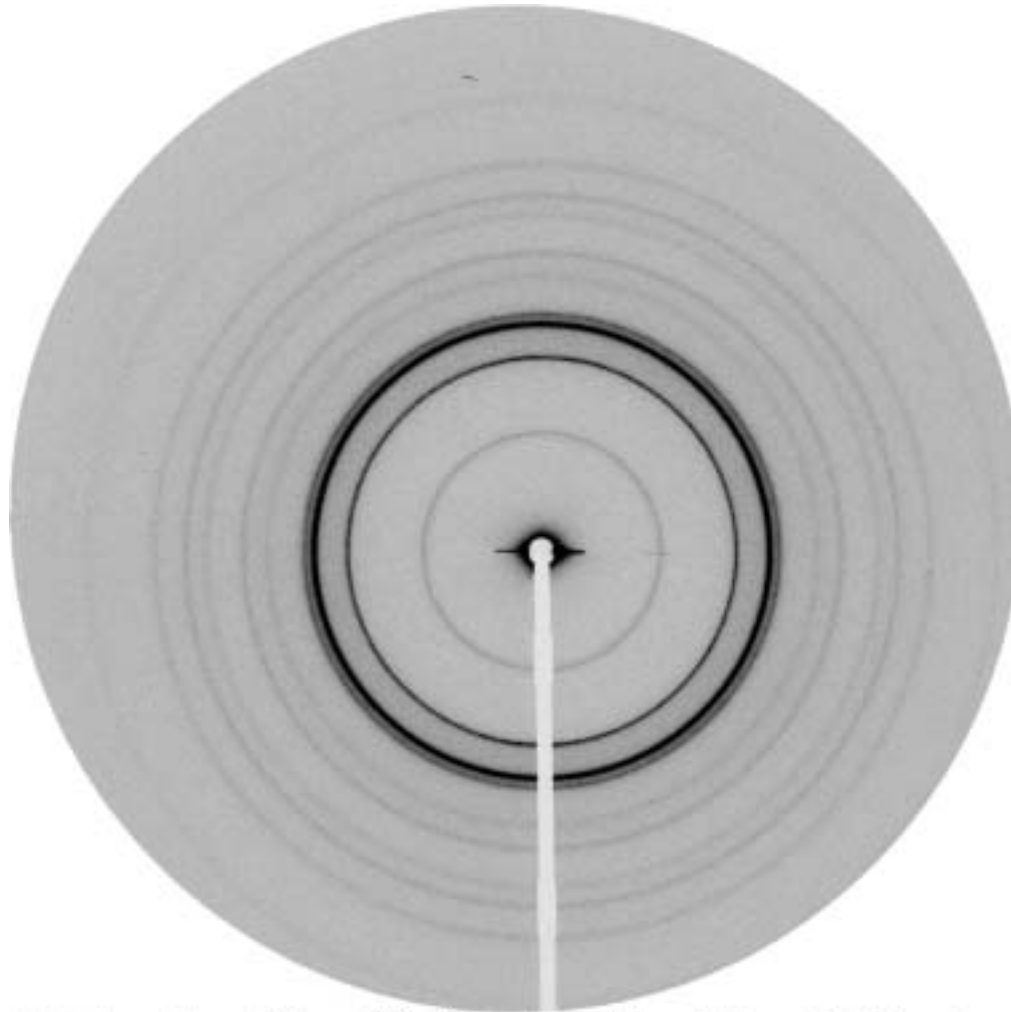
Fig. 2 The natural sequence of lyotropic liquid-crystalline phases *vs.* the average interfacial mean curvature. For a given lipid, the experimental parameter controlling the curvature can be any thermodynamic variable such as hydration, temperature, pressure, *etc.* Reproduced from *Seddon et al., Phys Chem Chem Phys* 2 (2000) 4485-4493.

In the regions labelled a, b, c, and d are found more complex intermediate phases, having 3-D periodicity. In the vast majority of cases these intermediate phases are of cubic symmetry: (a) inverse cubic micellar; (b) inverse cubic bilayer (bicontinuous); (c) cubic bilayer (bicontinuous); (d) cubic micellar.

Cubic and Hex Phases in +/- mix.

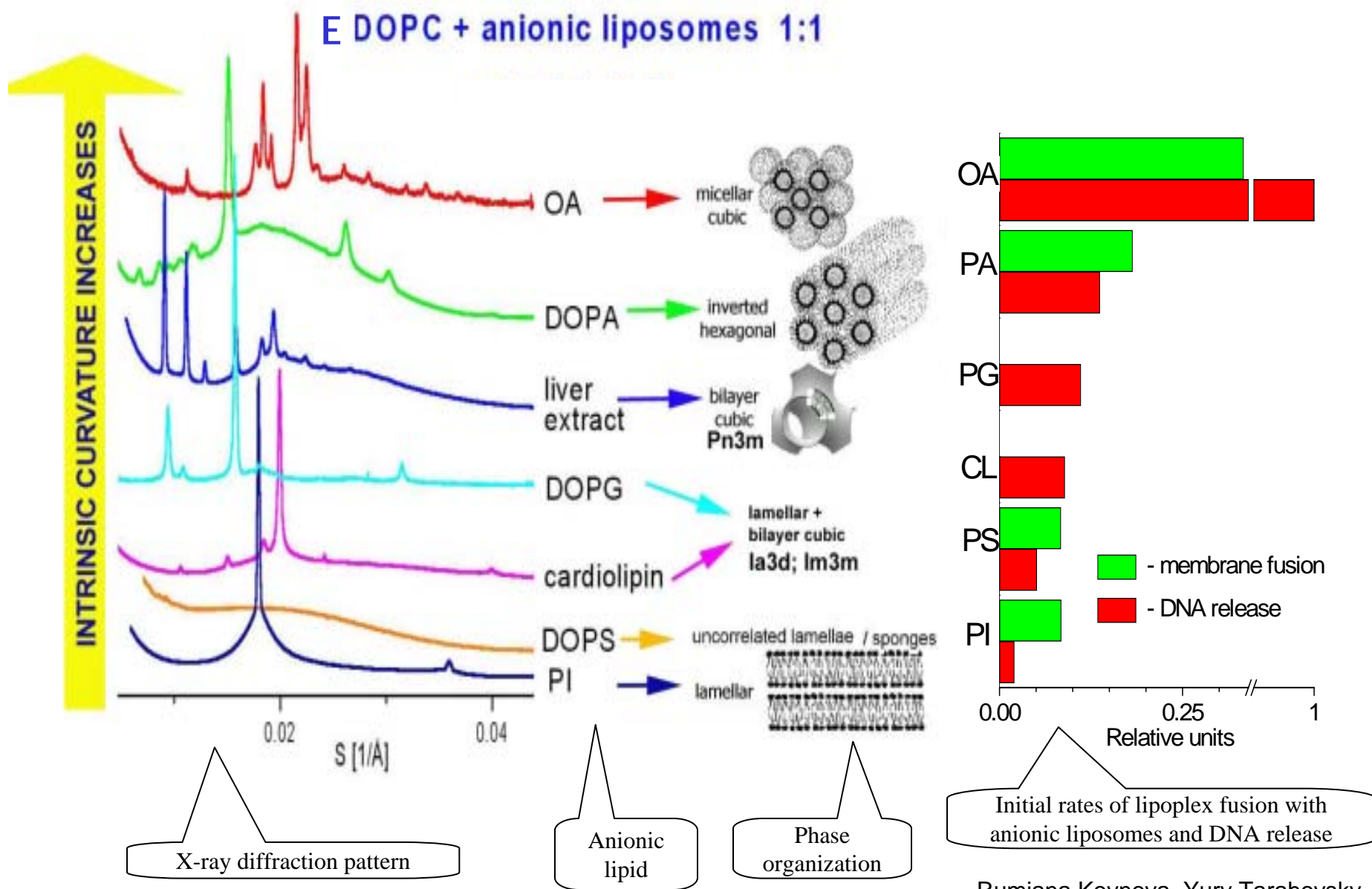


X-ray Diffraction patterns of lipids are distinctive to the phase

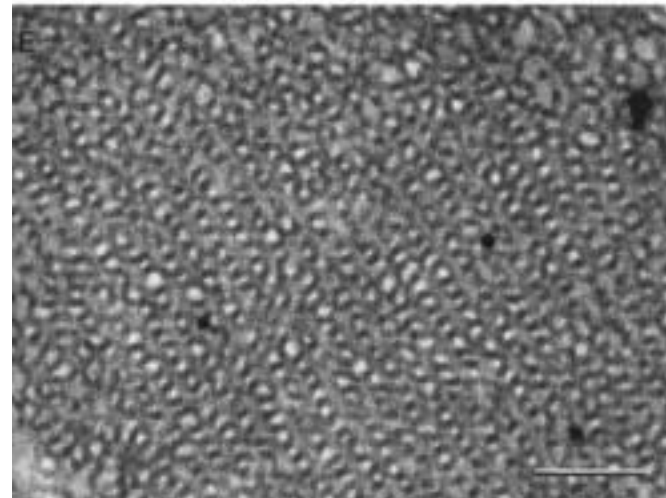
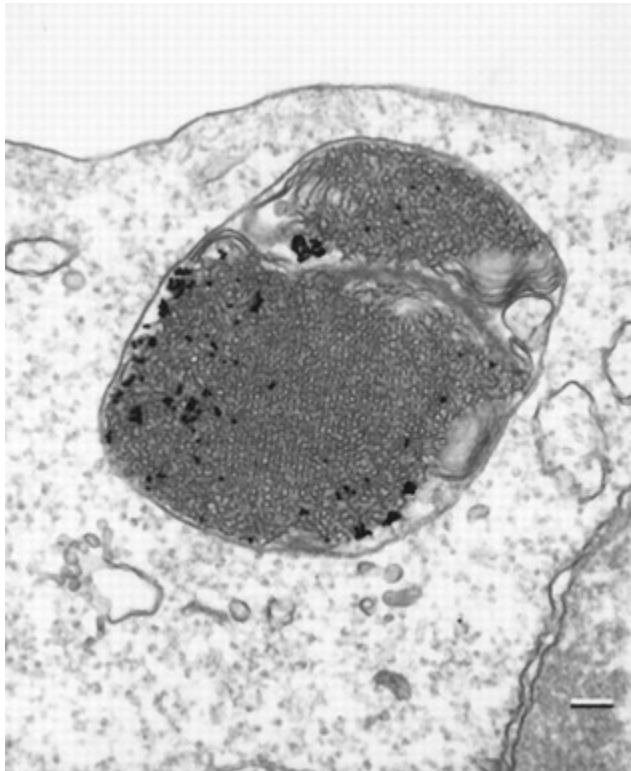


Typical small-angle X-ray diffraction pattern of the micellar cubic $Fd3m$ phase, from the EDLPC/EDOPC (6:4) / OA mixture.

CORRELATION BETWEEN INTRINSIC CURVATURE OF LIPIDS, MEMBRANE FUSION AND DNA RELEASE



Bilayer cubic arrays are seen in lipoplexes internalized in cells



Conclusions, Part I

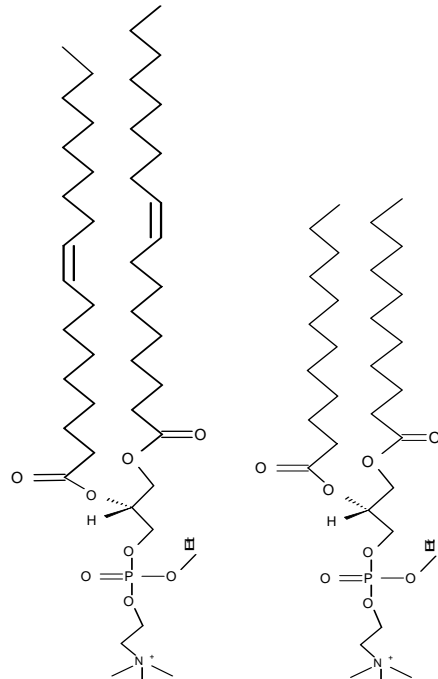
Anionic lipids:

- Fuse with cationic lipoids
- Cause dissociation of DNA from lipoplexes
- Generate non-lamellar phases when combined with cationic lipoids; the fusion rate and the extent of DNA release correlate with the intrinsic curvature of that phase

Part II. Mixed lipid lipoplexes

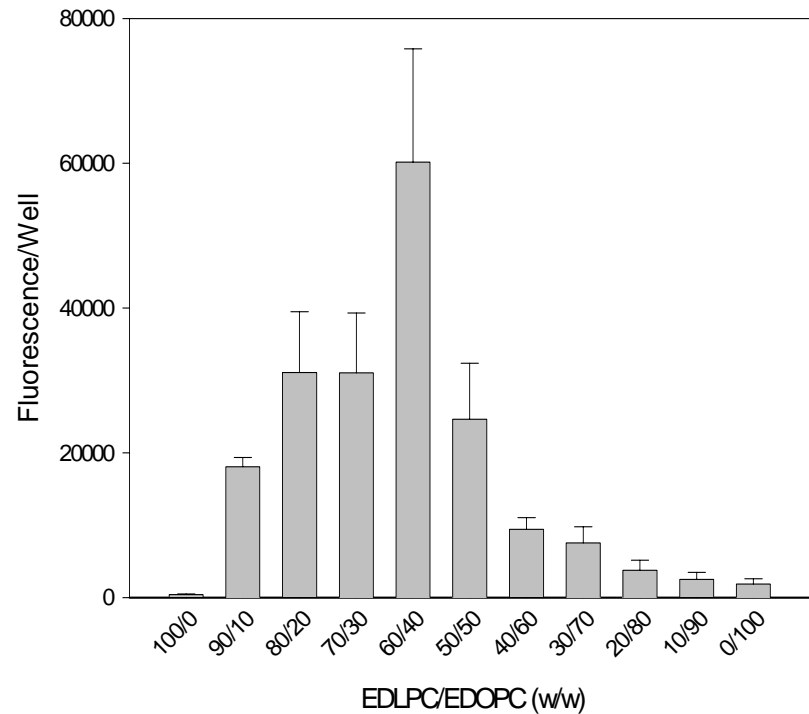
Mixtures of cationic lipids exhibit greater transfection activity, which seems to correlate with greater fusogenicity and greater tendency to generate high curvature phases when mixed with anionic lipids

Medium- and long-chain cationic lipoids act synergistically in transfection



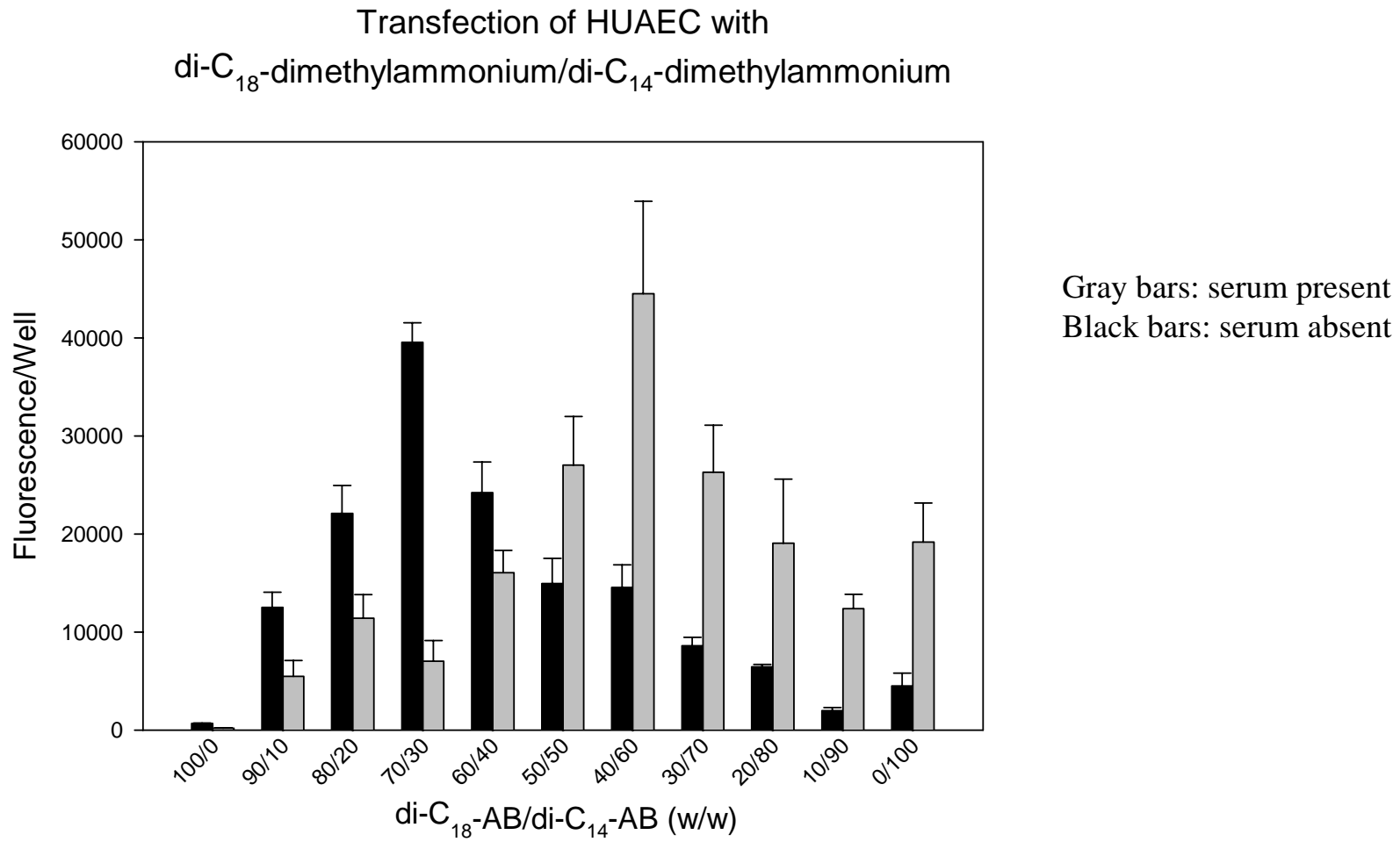
EDOPC

EDLPC

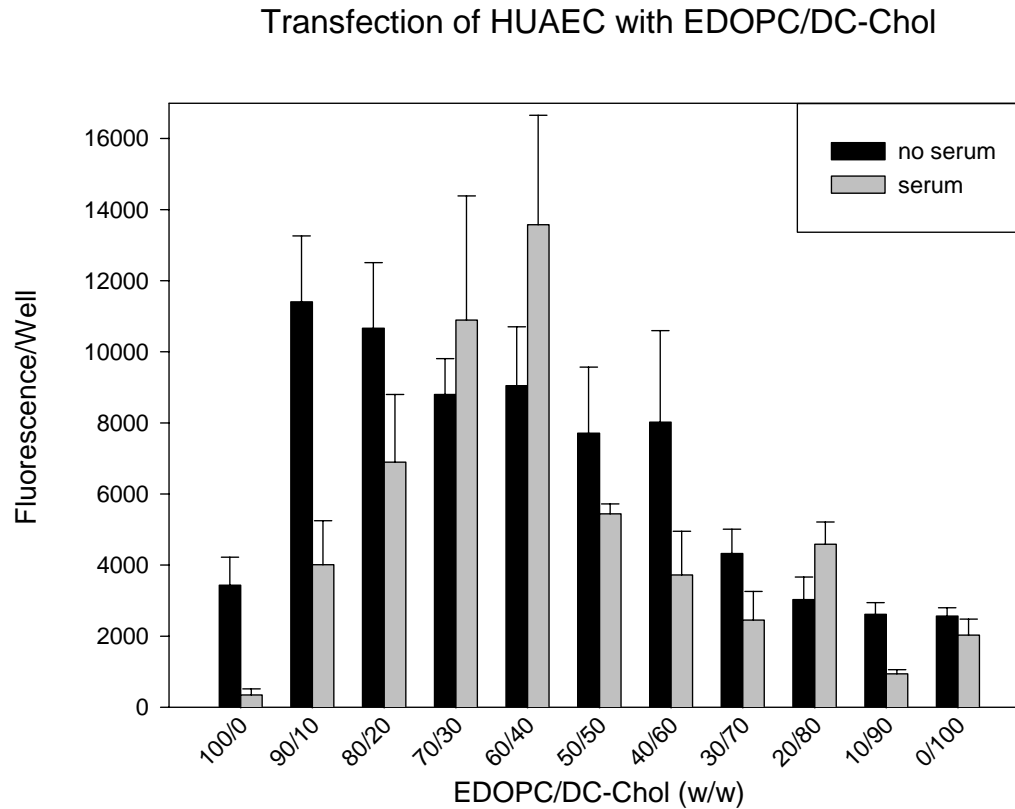


The mixed lipoid effect is general and
not limited to cationic
phosphatidylcholinium compounds.

Tetraalkylammonium compounds exhibit the mixed lipoid effect



EDOPC and cationic cholesterol exhibit the mixed lipoid effect

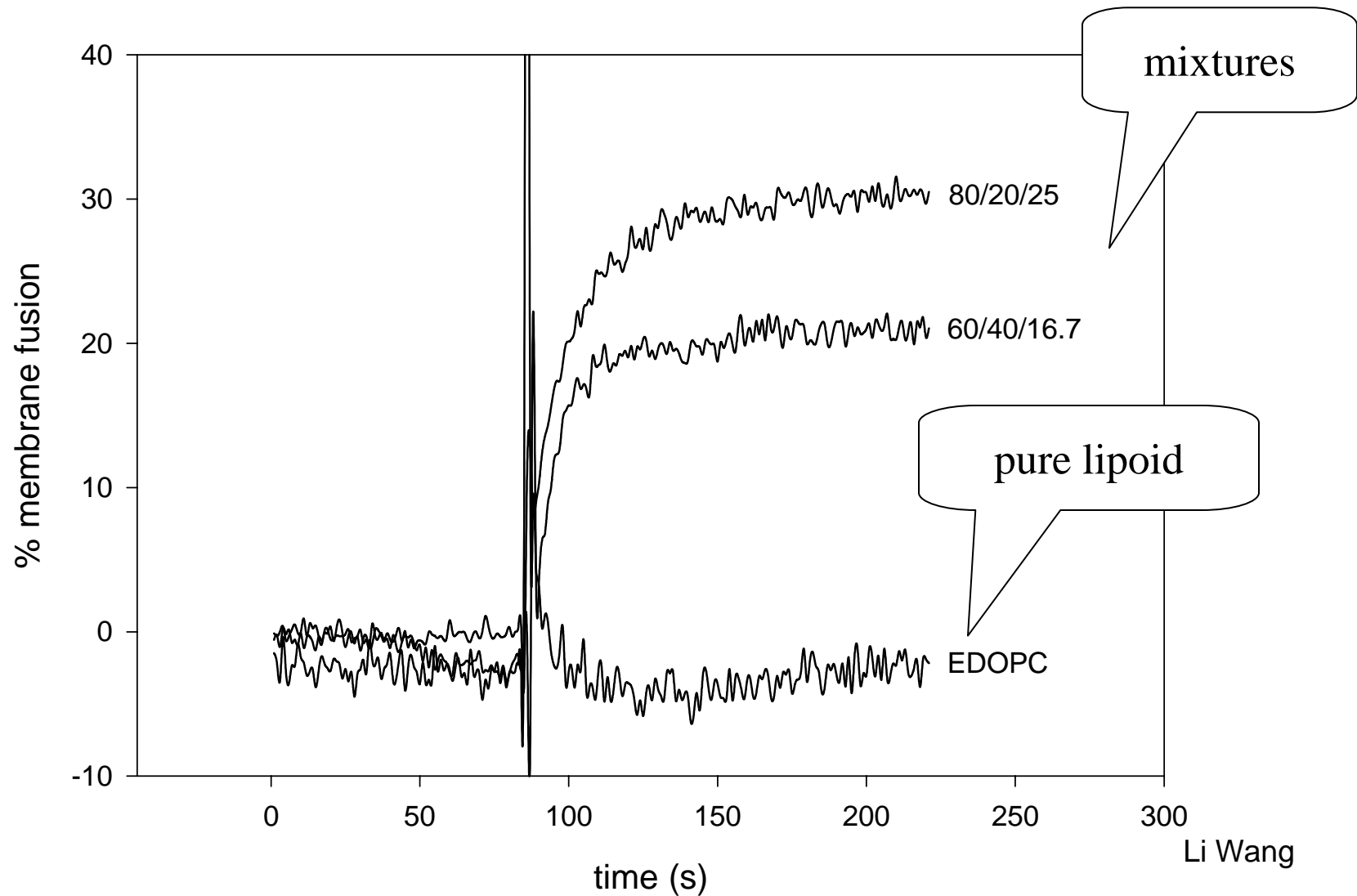


Do the shorter-chain lipoids
facilitate fusion/mixing of
lipoplex lipids with anionic lipid?

Yes.

(what did you expect?)

Lipid mixing of lipoplex lipid with DOPC bilayers containing 20% DOPG

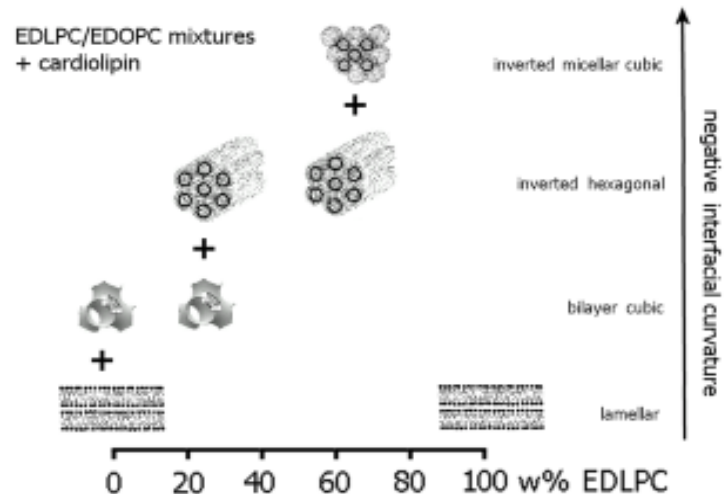
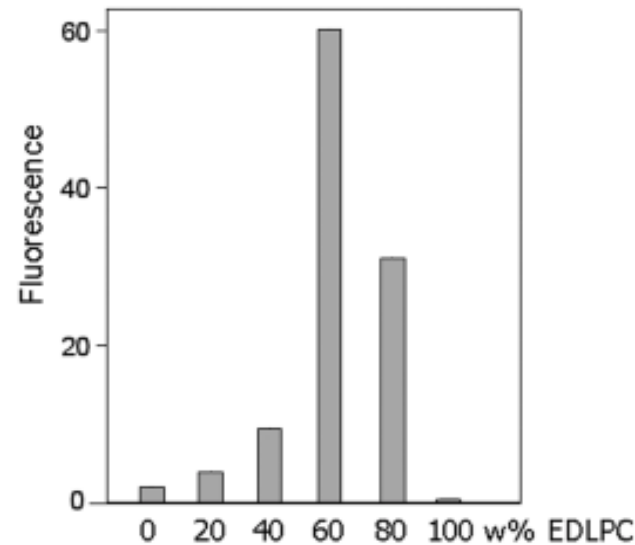


So, the mixed lipoids facilitate membrane fusion with anionic lipids.

Do they also promote non-bilayer phase formation when combined with anionic lipids?

Yes.

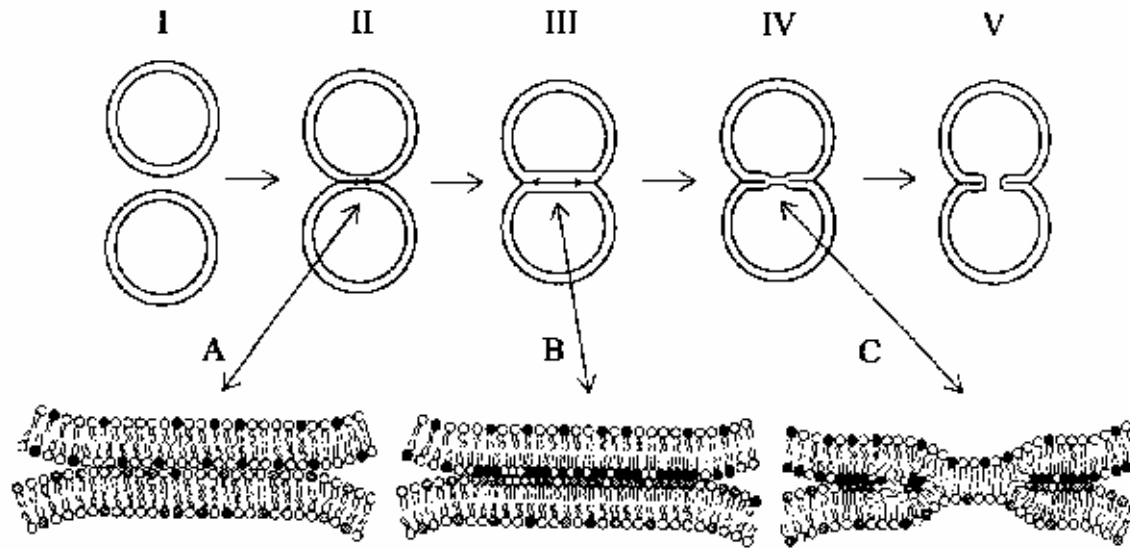
Inverted cubic micellar phases form when the optimal EDLPC/EDOPC mixture is combined with anionic lipid; this phase has the most negative curvature possible



MIXED CATIONIC PHOSPHOLIPOIDS FORM MICELLAR CUBIC PHASES IN COMBINATION WITH MANY ANIONIC LIPIDS WHEREAS THE PURE LONG CHAIN LIPID AND THE CATIONIC-PE COMBINATION FORM LAMELLAR OR HEXAGONAL PHASES

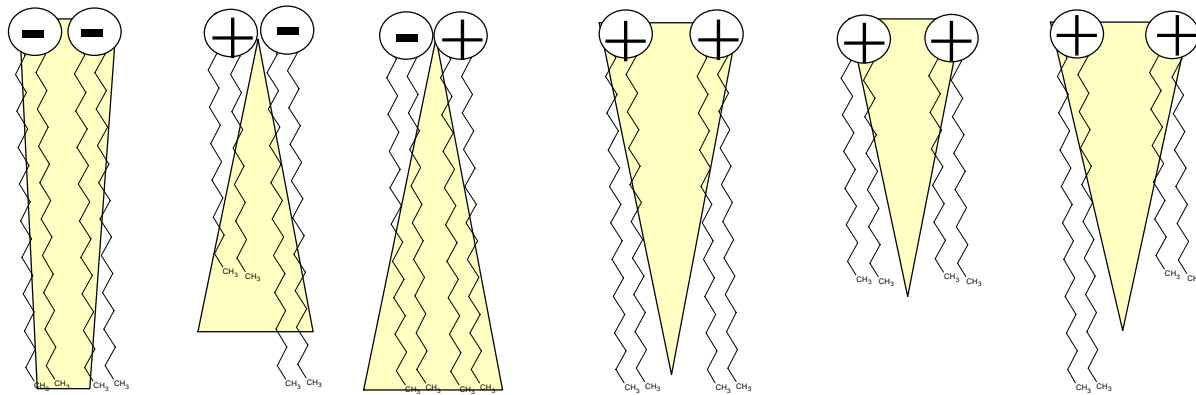
Cationic Anionic	C18PC	C12/C18 PC Mix 6:4	DOTAP/DOPE 1:1 Cationic/neutral
Oleic acid	Micellar cubic	Micellar cubic	Micellar cubic
DOPA	Hexagonal	Micellar cubic	Hexagonal + trace of cubic
DOPG	Lamellar + Bilayer cubic	Micellar cubic	Hexagonal + trace of cubic
Cardiolipin	Lamellar + Bilayer cubic	Micellar cubic	Hexagonal + trace of cubic
DOPS	Lamellar	unresolved	Hexagonal + trace of cubic

Why do mixtures of lipids with different tails favor fusion?



Fusion intermediates involve high curvature, particularly in the negative direction, and this can be provided for by a differential distribution of the two lipids in the monolayers of each bilayer

Why do mixtures of lipids with different tails favor non-lamellar phases?



Like fusion intermediates, non-lamellar phases are favored by lipids with different intrinsic curvatures, and after combining with anionic lipid, cationic mixtures can assume an even larger variety of shapes

That's all folks

Thank you

Appearance of micellar cubic phases in +/- mixtures (3d incub.)

Anionic	Cationic	EDOPC	EDLPC/EDOPC 6:4	DOTAP/DOPE 1:1
Oleic acid		micellar cubic Fd3m	micellar cubic Fd3m	micellar cubic Fd3m
Dioleoylphosphatidic acid (DOPA)		inverted hexagonal H _{II}	bilayer cubic Pn3m (but Fd3m in kinetic exps, after 20')	H _{II}
Dioleoylphosphatidylglycerol (DOPG)		lamellar + bilayer cubic Ia3d	micellar cubic Fd3m (also in kinetic exps, after 20')	H _{II} + Pn3m (trace)
Cardiolipin (heart) (CL)		lamellar + bilayer cubic Pn3m	micellar cubic Fd3m (in kinetic exps – micellar phase appears after 10')	H _{II} + Pn3m (trace)
Dioleoylphosphatidylserine (DOPS)		lamellar	unresolved (could be Fd3m – only 2 reflections, fitting)	H _{II} + Pn3m (trace)